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XLIII. EXAMINATION OF THE LLOYD METHOD FOR THE ASSAY OF ALKALOIDS.*

By Herbert T. Nichols and Thos. H. Norton.

In February, 1891, Prof. J. U. Lloyd read before the Cincinnati Section of the American Chemical Society a paper on the analysis of alkaloids, which was presented later before the American Pharmaceutical Association at its annual meeting, published in pamphlet form,† and printed more or less fully in several home and foreign journals. The method proposed, on account of its claims to rapidity and reasonable accuracy, evoked much criticism both friendly and adverse. The possession of a reliable method placing in the hands of the chemist the means of analyzing easily and quickly the majority of the members of the group of the alkaloids, is of such prime importance, that we undertook the following study of the process with the view of ascertaining as definitely as possible the limits of accuracy in the case of the leading alkaloids, under varying conditions.

The method proposed by Prof. Lloyd, and employed in all our experiments, is, briefly stated, as follows:

5 cc. of an alkaloidal solution are poured into a mortar, and a sufficient amount of a mixture of equal parts of ferric hydrate and sodium bicarbonate added to form a stiff paste. This magma is then triturated with 20 cc. of chloroform. Should so much alcohol be abstracted that the magma becomes pulverulent, a small amount of water or of a solution of glucose is added and triturated until a stiff paste is again formed, from which the chloform separates clearly with no trace of turbidity.

The chloroform extract is decanted into a small porcelain dish and evaporated.

The magma is then washed three times by trituration with chloroform. 10 cc. of chloroform are used each time and the washings are decanted as before and evaporated in the same dish.

The residue in the dish is covered with a 2 per cent. solution of sulfuric acid, digested for a few minutes on the water bath and filtered. The same diluted acid is used to wash the residue on the filter paper.

*Read before the Cincinnati Section of the American Chemical Society, Jan. 28, 1892.

†Copies of a revised edition of this pamphlet are gladly furnished to those interested in the matter, by application to the author, Prof. J. U. Lloyd, Court St., Cincinnati.

The clear solution of the sulfate of the alkaloid, thus obtained, is rendered slightly alkaline by ammonia, and rotated in a separating funnel with 10 cc. of chloroform for about two minutes. This operation is repeated four times, the chloroform being drawn off each time.

The solution of the alkaloid in chloroform is then evaporated to dryness in a small platinum dish or on a watch-glass over the water-bath. While still on the water-bath the residue is stirred with the sharp point of a knife, to ensure the total removal of the chloroform.

After cooling in the desiccator, the dish or watch-glass is weighed with its residue of pure alkaloid.

The chief advantage and essential peculiarity claimed for the process just described is the total avoidance of any emulsion in the chloroform extract from the magma obtained by the treatment with ferric hydrate and sodium bicarbonate at the outset. The remaining steps in the process are practically identical with those hitherto used in similar analytical determinations.

In the study of the method in question, we have extended our experiments over the following subjects, viz:

1. Purity of the chloroform and other reagents used.
2. Insolubility of ammonium sulfate in chloroform.
3. Completeness of the extraction of the alkaloids from the magma.
4. Completeness of the extraction of alkaloids from alkaline solution by rotary agitation with chloroform.
5. Complete assays of pure alkaloids and of their fluid extracts.
6. Increase in weight of certain alkaloids after solution in chloroform and subsequent evaporation.
7. Substitution of aluminum and chromium hydrates for ferric hydrate.
8. Limits of precaution to be observed in weighing.
9. Amounts of moisture present in pure commercial alkaloids.

A. Experimental Part by H. T. Nichols.

I. Purity of the Reagents Used.

Chloroform.—The purity of the chloroform used should be tested by evaporating about 10 cc. on the water-bath. If pure enough for the purpose in view, it should leave absolutely no residue.

This precautionary test is especially necessary in the case of chloroform which is not of recent manufacture. Where many determinations of this nature are made, a large proportion of the chloroform can be saved by evaporation beneath an inverted funnel leading to a condenser through which a current of air is drawn. If rubber connections are used, redistillation is of course, necessary.

Ferric hydrate and sodium bicarbonate.—These should be pure, or at least free from all substances capable of being dissolved by chloroform.

The reagents used in the following analyses were triturated with chloroform, and the decanted liquor left no residue.

II. INSOLUBILITY OF AMMONIUM SULFATE IN CHLOROFORM.

As no statements with regard to the solvent powers of chloroform on ammonium sulfate are on record, it seemed desirable to decide the question by actual experiment.

Several grams of ammonium sulfate were triturated with 20 cc. of chloroform, and filtered. The filtrate evaporated to dryness on the water-bath left no weighable residue. This experiment was repeated five times with the same result.

10 cc. of a 2 per cent. solution of sulfuric acid were made alkaline by ammonia and agitated with 20 cc. of chloroform, in a separating funnel. The chloroform was then drawn off and evaporated to dryness on the water-bath. This was repeated five times, likewise without obtaining any residue.

Insolubility of ammonium acetate in chloroform.—In this connection a few experiments were made to ascertain whether ammonium acetate is soluble in chloroform.

10 cc. of acetic acid were made alkaline by ammonia and agitated in a separating funnel, with 20 cc. of chloroform.

The chloroform was then drawn off and evaporated to dryness over the water-bath on a watch-glass. This operation was repeated five times, and gave in no instance a residue. The experiment was altered, by allowing a few drops of the solution of ammonium acetate to run through with the chloroform. This when evaporated to dryness yielded no residue. The operation was repeated five times with like results.

The observation has already been made that ammonium acetate is decomposed at 100° C.

This observation was confirmed by three experiments in which solutions of ammonium acetate were evaporated to dryness, and left no residue.

III. COMPLETENESS OF THE EXTRACTION OF ALKALOIDS FROM THE MAGMA BY CHLOROFORM.

Three series of tests were made to ascertain whether any appreciable amount of alkaloid remained in the magma after four extractions with chloroform.

Several magmas containing nux vomica were extracted in the usual manner.

In no case was the intensely bitter taste peculiar to this alkaloid perceptible after the third washing with chloroform.

A weighed amount of cinchonin, one part of which is soluble in over three hundred parts of chloroform, was treated as before.

The residue obtained from the chloroform extract was almost equal to the amount used, showing that even in the case of this difficultly soluble alkaloid, scarcely any portion was retained by the magma.

A further test of the delicacy of the process was shown by the following experiment:

0.00001 gm. of a mixture of brucin and strychnin was put through the assay and the residue on the watch-glass, though not visible, still yielded the bitter taste of the alkaloids.

IV. COMPLETENESS OF THE EXTRACTION OF ALKALOIDS FROM ALKALINE SOLUTIONS BY ROTARY AGITATION WITH CHLOROFORM.

Experiments were made to find the number of rotations necessary for the complete extraction of an alkaloid when the solution of the alkaloid and ammonium sulfate is rotated with chloroform. 1 gm. of an alkaloid was dissolved in acidulated water, and the solution was made up to 100 cc. or a 1 per cent. solution. 10 cc. of this solution were used each time, containing in each case, therefore, 0.1 gm.

Each portion was rendered slightly alkaline by the addition of ammonia, put into a separating funnel, and rotated with chloroform. The dried residue obtained from the chloroform drawn off after each period of rotation was weighed separately.

The rotations were made with the circling motion suggested in his article by Prof. Lloyd, and precautions were observed to pre-

vent the liquids from being shaken together. The average time of each period of rotation was about two minutes.

Caffein.—Amount used, .1 gm.

Amount Recovered after each Successive Extraction.

	First Rotation.	Second Rotation.	Third Rotation.	Fourth Rotation.	Total Amount Recovered.
A	.0886 gm.	.0048 gm.	.0016 gm.	.0000 gm.	.0950 gm.
B	.0826 "	.0120 "	.0032 "	.0006 "	.0944 "
C	.0708 "	.0222 "	.0048 "	.0006 "	.0984 "
D	.0846 "	.0092 "	.0020 "	.0000 "	.0958 "
E	.0832 "	.0098 "	.0024 "	.0006 "	.0960 "
Average,	.0820 "	.0096 "	.0028 "	.0004 "	.0959 "

The greatest loss occurred in *B*, .0056 gm. The least loss was in *C*, .0016 gm. The average loss was .0140 gm., or 4 per cent.

Brucin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A	.1170 gm.	.0084 gm.	.0008 gm.	.1262 gm.
B . . .	.1104 "	.0040 "	.0000 "	.1152 "
C	.1026 "	.0042 "	.0004 "	.1072 "
D	.1038 "	.0036 "	.0000 "	.1068 "
E	.1004 "	.0036 "	.0000 "	.1040 "
Average,	.1068 "	.0048 "	.0002 "	.1120 "

Average total gain, 12 per cent.
The variations in total gain ranged from 4 to 26.2 per cent.

It will be noticed that the total amount has increased over the original amount 12 per cent. on an average. As will be seen later in the section on the increase in weight of certain alkaloids after treatment with chloroform, this result was to be expected.

Strychnin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A	.0810 gm.	.0032 gm.	.0000 gm.	.0842 gm.
B	.0948 "	.0026 "	.0000 "	.0974 "
C	.0898 "	.0016 "	.0000 "	.0914 "
D	.0840 "	.0024 "	.0000 "	.0864 "
E	.0906 "	.0028 "	.0002 "	.0936 "
Average	.0880 "	.0025 "	.0000 "	.0906 "

Average total loss, 9.4 per cent.
The variations in the total loss ranged from 2.6 to 15.8 per cent.

Atropin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A . . .	.0926 gm.	.0032 gm.	.0000 gm.	.0958 gm.
B	.0786 "	.0116 "	.0032 "	.0934 "
C	.0928 "	.0024 "	.0000 "	.0952 "
D	.0916 "	.0030 "	.0000 "	.0946 "
E	.0890 "	.0062 "	.0010 "	.0952 "
Average,	.0889 "	.0053 "	.0008 "	.0948 "

Average total loss, 5.2 per cent.

The variations in total loss ranged from 4.2 to 6.6 per cent.

Aconitin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A	.0960 gm.	.0040 gm.	.0006 gm.	.1006 gm.
B	.1066 "	.0042 "	.0002 "	.1110 "
C	.0998 "	.0036 "	.0004 "	.1038 "
D	.0926 "	.0036 "	.0002 "	.0964 "
E	.0923 "	.0030 "	.0000 "	.0966 "
Average,	.0977 "	.0037 "	.0003 "	.1017 "

Average total gain, 1.7 per cent.

The variations in the total loss or gain ranged from 3.6 per cent. loss to 11 per cent. gain. The latter high figure, obtained in but instance, is to be considered as probably due to error.

Quinin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A	.0996 gm.	.0052 gm.	.0000 gm.	.1048 gm.
B	.0974 "	.0022 "	.0002 "	.0998 "
C	.0990 "	.0026 "	.0002 "	.1018 "
D	.0966 "	.0040 "	.0006 "	.1012 "
E	.0954 "	.0020 "	.0000 "	.0974 "
Average,	.0976 "	.0032 "	.0002 "	.1010 "

Average total gain, 1.2 per cent.

The variations in the total loss or gain ranged from 2.6 per cent. loss to 4.8 per cent. gain.

Cinchonin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A	.0796 gm.	.0156 gm.	.0003 gm.	.0955 gm.
B	.0862 "	.0070 "	.0004 "	.0936 "
C	.0836 "	.0036 "	.0006 "	.0878 "
D	.0762 "	.0112 "	.0006 "	.0880 "
E . . .	.0802 "	.0114 "	.0004 "	.0920 "
Average	.0812 "	.0102 "	.0005 "	.0919 "

Average total loss 8.1 per cent.
The variations in the total loss ranged from 4.5 to 12.2 per cent.

Cinconin is soluble with difficulty in chloroform, and therefore the amount of alkaloid obtained in the first rotation is less than in the case of other alkaloids.

Cinchonidin.—Amount used, .1 gm.

Amount Recovered after each Successive Rotation.

	First Rotation.	Second Rotation.	Third Rotation.	Total Amount Recovered.
A	.0950 gm.	.0036 gm.	.0004 gm.	.0990 gm.
B	.0924 "	.0026 "	.0004 "	.0954 "
C	.0924 "	.0024 "	.0006 "	.0954 "
D	.0926 "	.0010 "	.0000 "	.0936 "
E	.0890 "	.0012 "	.0000 "	.0902 "
Average	.0923 "	.0022 "	.0003 "	.0948 "

Average total loss, 5.2 per cent.
The variations in the total loss ranged from 1 to 9.8 per cent.

SUMMARY.

The highest average gain was in the case of brucin, amounting to 12 per cent.; and the lowest average gain was in the case of quinin, amounting to 1.2 per cent. The lowest average loss was in the case of caffeïn, amounting to 4 per cent., and the highest average loss was in the case of strychnin, amounting to 9.4 per cent. A fourth agitation yielded in but few instances a weighable amount.

V. COMPLETE ASSAYS OF FLUID EXTRACTS, OF THEIR PURE ALKALOIDS AND OF MIXTURES OF BOTH.

As the method in question is of special applicability to the analysis of fluid extracts, a study was made of the degree of uniformity attainable in this field.

Fluid extracts are alcoholic or hydro-alcoholic solutions of the soluble constituents of vegetable products, those of alkaloidal drugs containing the alkaloids in natural combination.

To test the method most thoroughly, parallel determinations were made, first of a fluid extract, next of the same fluid extract with a known amount of its proper alkaloid added, and thirdly of a standard solution of the alkaloid dissolved in alcohol and water.

In this manner the fluid extracts and alkaloids of nux vomica, belladonna, guarana, ipecac, aconite root, and henbane were assayed. The fluid extracts used were those obtained in commerce.

Nux Vomica.—Five samples of the same fluid extract were analyzed:

Percentages Obtained.

I.	II.	III.	IV.	V.	Average.
1.39%	1.50%	1.45%	1.46%	1.39%	1.44%

.125 gm. of strychnin and .125 gm. of brucin were added to 50 cc. of the fluid extract, and 5 cc. were used in each assay. The results were as follows:

Percentages Obtained.

I.	II.	III.	IV.	V.	Average.	Theory.
2.03%	1.98%	1.95%	2.00%	2.00%	1.99%	1.94%

The increase in amount is due to the property shown by brucin of increasing in weight after treatment with chloroform. See paragraph VI.

.75 gm. of the mixture of pure brucin and strychnin was dissolved in 50 cc. of alcohol and water, making a 1.5 per cent. solution.

The results of the analysis were as follows:

Percentages Obtained.

I.	II.	III.	IV.	V.	Average.	Amount Used.
1.60%	1.51%	1.48%	1.47%	1.52%	1.52%	1.50%

Average increase, .02 per cent., or 1.3 per cent. of the alkaloid used.

Belladonna.—The fluid extract of belladonna was assayed with the following results, all from the same sample:

Percentages Obtained.

I.	II.	III.	IV.	V.	Average.
0.50%	0.55%	0.54%	0.52%	0.50%	0.52%

The greatest variation from the average was .03 per cent., or 6 per cent. of the weight of the alkaloid.

To 100 cc. of the same sample of fluid extract, 1 gm. of pure atropin was added, and the following results were obtained:

Percentages Obtained.

I.	II.	III.	IV.	V.	Average.	Theory.
1.49%	1.46%	1.46%	1.49%	1.52%	1.48%	1.52%

In analyzing the pure alkaloid, it was found that a solution of atropin in alcohol and water deteriorated so rapidly that the same solution could not be used for comparative assays. The atropin was therefore weighed directly each time. The results were as follows:

Per Cent. of Atropin Used.

I.	II.	III.	IV.	V.
1.12%	1.20%	1.49%	1.39%	0.88%

Per Cent. of Atropin Obtained.

I.	II.	III.	IV.	V.
1.04%	1.15%	1.46%	1.36%	0.88%

Guarana.—The fluid extract of guarana can be analyzed by direct determination from the chloroform extract obtained from the magma. The results were as follows:

I.	II.	III.	IV.	V.	Average.
3.84%	3.86%	3.84%	3.71%	3.78%	3.81%

1 gm. of caffeïn was dissolved in 50 cc. of the fluid extract of guarana, and the strengthened extract was assayed with the following results:

I.	II.	III.	IV.	V.	Average.	Theory.
5.68%	5.66%	5.56%	5.52%	5.40%	5.56%	5.81%

The average loss is 4 per cent. of the alkaloid.

4 gms. of caffeïn were dissolved in 100 cc. of alcohol and water, forming a 4 per cent. solution, which was analyzed as follows:

I.	II.	III.	IV.	V.	Average.	Theory.
3.82%	3.76%	3.80%	3.83%	3.86%	3.81%	4.00%

Average loss, .19 per cent., or 4.7 per cent. of the alkaloid used

Ipecac.—A fluid extract of ipecac gave the following results:

I.	II.	III.	IV.	V.	Average.
1.42%	1.37%	1.52%	1.47%	1.52%	1.46%

In this case the greatest departure from the average was 7 per cent. of the weight of alkaloid present.

The alkaloid emetin is of such a delicate and unstable nature

that it was considered useless to carry out the check experiments employed in the cases of the preceding alkaloids.

Aconite Root.—The assays of the fluid extract of aconite root gave the following results:

I.	II.	III.	IV.	V.	Average.
0.41%	0.42%	0.41%	0.44%	0.42%	0.42%

In these analyses the greatest variation is 5 per cent. of the alkaloid present.

It was not deemed expedient to make experiments with alkaloidal addition to the fluid extract of aconite root or of hyoscyamus, on account of the instability of aconitin and hyoscyamin.

Hyoscyamus.—The fluid extract of hyoscyamus was assayed as follows:

I.	II.	III.	IV.	V.	Average.
0.10%	0.10%	0.09%	0.09%	0.10%	0.10%

VI. INCREASE IN WEIGHT OF CERTAIN ALKALOIDS AFTER SOLUTION IN CHLOROFORM AND SUBSEQUENT EVAPORATION.

An interesting and important fact noticed in connection with this work was the alteration in weight of some alkaloids after solution in chloroform, and subsequent evaporation. This alteration, usually a gain in weight, was most noticeable in the case of brucin.

The real nature of this change was not ascertained. As, however, it has a marked bearing on the value of the process in question, a series of experiments as to the extent of this variation was carried out in the following manner.

A weighed amount of an alkaloid varying from 0.1 to 0.01 gm. was dissolved in chloroform, the solution placed upon a tared watch-glass, and then evaporated on the water-bath to dryness. In the case of those alkaloids which were left in a gummy condition, the residues were stirred with the point of a pen-knife.

The glass was then cooled in a desiccator, over calcium chlorid, for about ten minutes, and quickly weighed.

Caffeïn.—Caffein, unlike most alkaloids, leaves a crystalline residue. The variations noted were inconsiderable, except where small amounts were used.

The figures obtained were as follows:

Percentages of Loss or Gain.

Amount of Caffeïn Used.	I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	0. %	0. %	0. %	—0.8%	—0.6%	—0.3%
0.05 " . . .	—1.9	—2.9	0.	—0.9	—2.1	—1.5
0.01 " . .	0.	0.	—3.0	0.	11.	+1.6

Brucin.—The results obtained were as follows:

Percentages of Gain in Weight.

Amount of Brucin Used.	I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	6.6%	9.3%	9.8%	9.4%	8.9%	8.9%
0.05 " . . .	8.3	6.6	6.3	9.8	11.0	8.4
0.01 " . . .	6.3	3.2	4.4	10.0	4.9	5.5
						Average, 7.6%

It will be noticed that the range of variation increases as the amount of the alkaloid used in the evaporation decreases.

Strychnin.—The results obtained were as follows:

Percentages of Loss or Gain.

Amount of Strychnin Used.	I.	II.	III.	IV.	V.	Average.
0.1 gm. . .	0.9%	—6.5%	—4.7%	—1.3%	2.7%	—1.8%
0.05 " . . .	0.0	—1.6	0.0	—0.7	1.3	0.4
						Average, —0.7%

Aconitin.—The results obtained were as follows:

Percentages of Gain.

Amount of Aconitin Used.	I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	6.7%	5.8%	4.1%	5.0%	5.9%	5.5%
0.05 " . . .	1.7	8.0	2.0	1.3	2.4	3.1
0.01 " . . .	0.0	2.0	2.6	1.9	3.0	1.7
						Average, 3.1%

Atropin.—The results obtained were as follows:

Percentages of Gain.

Amount of Atropin Used.	I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	0.3%	0.2%	0.2%	0.4%	2.2%	0.6%
0.05 " . . .	0.7	0.0	1.5	1.0	1.8	1.0
0.01 " . . .	0.0	4.0	*13.0	0.0	0.0	2.4
						Average, 1.3%

*Evidently an error.

Quinin.—The results obtained were as follows:

Amount of Quinin Used.	Percentages of Loss or Gain. I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	0.4%	—0.2%	1.1%	3.4%	1.3%	1.2%
0.05 " . . .	—0.7	0.0	7.1	0.0	1.5	1.6
0.01 " . . .	0.0	0.0	1.9	0.0	3.3	1.0
						Average, 1.3%

The average gain is comparatively slight, although the variations range from —0.7 per cent. to +7.1 per cent.

Cinchonin.—The results obtained were as follows:

Amount of Cinchonin Used.	Percentages of Loss or Gain. I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	0.0%	0.0%	—0.5%	0.0%	0.0%	—0.1%
0.05 " . . .	0.7	1.9	1.7	0.0	1.9	1.2
0.01 " . . .	0.0	0.0	0.0	0.0	—2.0	—0.4
						Average, 0.1%

Cinchonidin.—The results obtained were as follows:

Amount of Cinchonidin Used.	Percentages of Gain. I.	II.	III.	IV.	V.	Average.
0.1 gm. . . .	0.0%	0.4%	0.4%	0.0%	0.2%	0.2%
0.05 " . . .	0.0	0.4	0.4	1.1	1.9	0.7
0.01 " . . .	7.9	2.2	0.0	0.0	5.7	3.1
						Average, 1.3%

VII. SUBSTITUTION OF ALUMINUM OR CHROMIUM HYDRATES FOR FERRIC HYDRATE.

Experiments were made with both of these substances to test their availability as reagents in this process, and it was found that if for any reason it is desirable to avoid the presence of iron, either aluminum or chromium hydrate can easily be used as a substitute, both making a good magma and rendering the tannate insoluble. Neither of these substances offers any especial advantage, however, over the ferric hydrate, which is also preferable on account of its cheapness.

VIII. LIMITS OF NECESSARY PRECAUTION IN WEIGHING.

In order to note the difference in weight between cooling in a desiccator and in the open air, several fluid extracts were assayed;

the residues obtained at 100° on a tared watch-glass, were cooled in a desiccator, and weighed. They were then reheated and cooled for twenty minutes in the open air, and again weighed. Again the same residues were heated and cooled for one hour in the air and weighed. The results were as follows:

Nux vomica,	cooled in the desiccator	1.388	per cent.
"	" 20 minutes in the air	1.392	"
"	" 1 hour in the air	1.396	"
Belladonna,	" in the desiccator	0.568	"
"	" 20 minutes in the air	0.568	"
"	" 1 hour in the air	0.572	"
Aconite root,	" in the desiccator	0.380	"
"	" 20 minutes in the air	0.380	"
"	" 1 hour in the air	0.380	"

The alkaloids of ipecac and henbane seemed to be more or less decomposed by reheating.

IX. MOISTURE IN VARIOUS COMMERCIAL ALKALOIDS.

The pure alkaloids used in the preceding experiments bore the labels of reputable manufacturers, and were found to contain variable amounts of moisture, which were either removed or deducted in the calculation.

These amounts were as follows:

	Moisture Present.	
Atropin	0.11	per cent.
Strychnin	1.29	"
Brucin	9.22	"
Caffeïn	0.05	"
Quinin	2.02	"
Cinchonin	0.94	"
Cinchonidin	0.20	"

B. Summary of Results by T. H. Norton.

The importance of possessing a reliable method for the rapid determination of alkaloids in solution is such that I have followed with great interest the work detailed in the preceding pages. The data there recorded are what may ordinarily be expected from the use of the Lloyd process in the hands of one fairly expert in the usual methods of quantitive analysis, and do not, of necessity, represent adequately the degree of accuracy or uniformity to be

counted upon when the process is employed by those less familiar with chemical manipulation, as in the case of the average pharmacist or physician. All results obtained were recorded in order to afford a correct average representation of the working of the method, and this fact will explain the presence of a few abnormal deviations in certain series, which would naturally be eliminated from consideration in establishing averages.

The data here given afford valuable material for estimating the accuracy of the process as one worthy of being incorporated among our standard methods of analysis, and for defining the limitations of its useful application. Its widest field would naturally be in the analysis of alkaloidal preparations for medicinal use, as well as in the assay of crude drugs, and these data may, therefore, be useful to those engaged upon the revision of the National Pharmacopoeia. Any recommendation in this direction would scarcely be in place here.

With regard to the applicability of the method, it is to be noted that it does not aim to separate one alkaloid from another, that its sole purpose is to determine gravimetrically the amount of non-volatile alkaloid or alkaloids, soluble in chloroform (or ether) present in a drug or solution, such as a fluid extract. In passing judgment upon the utility of the method, it is further necessary to bear in mind the varying tendencies of the alkaloids to undergo decomposition. Time, light, temperature, the nature of solvents, all are factors in producing more or less deterioration in the great majority of members of this class.

Keeping these facts in view, the results of this investigation may be briefly summarized as follows:

1. The manipulation involved in the method is exceedingly simple, and fairly uniform results should be obtained by one familiar with elementary quantitative work.

2. Absolutely pure chloroform must be used. The three hydroxides, $Fe_2(OH)_6$, $Cr_2(OH)_6$ and $Al_2(OH)_6$, may be used indifferently in the production of a magma.

3. Ammonium sulfate is totally insoluble in chloroform.

4. The completeness of the extraction of alkaloids from the ferric magma by means of chloroform is all that the most rigid analyst can demand. This was proved gravimetrically in the case of

the difficultly soluble cinchonin, and by means of the sense of taste with emetin, brucin and strychnin.

5. The extraction of alkaloids from an alkaline solution in the presence of ammonium sulfate, by means of chloroform, is complete after three treatments, and very nearly so after two in the cases of brucin, strychnin, atropin, aconitin, quinin, cinchonin and cinchonidin, while an additional treatment is necessary in the case of caffeïn.

6. The *recovery* of alkaloids under the conditions given in the above paragraph is somewhat variable, as will be seen from this table summarizing the losses and gains, and arranged according to the range of variation, beginning with the least variable.

Amount Recovered from 100 Parts in a Series of Five Analyses.

Alkaloid Used.	Lowest.	Highest.	Average.
Atropin	93.4	95.8	94.8
Caffeïn	94.4	98.4	95.9
Quinin	97.4	104.8	101.0
Aconitin*	96.4	103.8	100.2
Cinchonin	87.8	95.5	91.9
Cinchonidin	90.2	99.0	94.9
Brucin*	104.0	115.2	109.6
Strychnin	84.2	97.4	90.6

In several cases, as in those of brucin, aconitin and quinin, an increase is due to the treatment with chloroform, as will be explained in paragraph 7. In all other instances, there is more or less loss, accompanied by variation in the results, ranging from 2.4 per cent. in the case of atropin, to 11.2 per cent. in that of brucin.

It is evident that the chief element of uncertainty in the use of this method is to be encountered at this stage. The difficulties arising from the variable deterioration of the alkaloids have not been entirely evercome in any process for their determination of extended application, and there is abundant room for further investigation in this field.

7. The extent to which simple solution in chloroform, and subsequent evaporation on the water-bath, affect the result, was made the subject of a special study, which gave the following figures:

*A single exceptionally high result is left out of consideration as being undoubtedly due to error.

Amounts Recovered from 100 parts in a Series of Five Analyses, in each of which 0.1 gm. was used.

Alkaloid Used.	Lowest.	Highest.	Average.
Atropin	100.2	102.2	100.6
Caffeïn	99.2	100.0	99.7
Quinin	99.8	103.4	101.2
Aconitin	104.1	106.7	105.5
Cinchonin	99.5	100.0	99.1
Cinchonidin	100.0	100.4	100.2
Brucin	106.6	109.8	108.9
Strychnin	93.5	102.7	98.2

Strychnin exhibits great variation with a tendency to loss. Caffeïn, cinchonin and cinchonidin, are apparently unaltered. Atropin and quinin gain slightly in weight, aconitin more so, and brucin most of all. The range of variation, except in the case of strychnin, is much more limited than in the preceding table. This increase of weight, as the result of treatment with chloroform, is an interesting and important fact, and a more extended study of its real nature is reserved for a later paper. The results summarized in this paragraph, applied as a correction to those given in paragraph 6, show the following average deterioration of the alkaloids, due to the first stages of the process, viz., from the formation of the magma to the final extraction with chloroform :

	Deterioration.	
Atropin	5.8 per cent.	
Caffeïn	4.4 "	
Quinin	0.2 "	
Aconitin	5.3 "	
Cinchonin	7.2 "	
Cinchonidin	5.3 "	
Brucin	.7 "	gain.
Strychnin	7.6 "	

It will be seen that quinin and brucin are the two alkaloids in the list, least liable to decomposition, while the average deterioration of the other six alkaloids ranges from 4.4 per cent. to 7.6 per cent.

8. The results obtained by the direct analysis of fluid extracts are tabulated as follows, and show the degree of uniformity to be expected in the application of the method to several of the ordinary alkaloidal solutions of commerce :

Percentages in a Series of Five Determinations.

Alkaloidal Extract.	Lowest.	Highest.	Average.
Nux vomica	1.39	1.50	1.44
Belladonna	.50	.54	.52
Guarana	3.71	3.86	3.81
Aconite	.41	.44	.42
Hyoscyamus	.09	.10	.097
Ipecac	1.37	1.52	1.46

Three of these same solutions reinforced with additional known amounts of the corresponding alkaloids in the pure state, gave the following results:

Percentages in a Series of Five Determinations.

	Lowest.	Highest.	Average.	Theory.
Nux vomica plus a mixture of equal amounts of brucin and strychnin	1.95	2.03	1.99	1.94
Belladonna plus atropin	1.46	1.52	1.48	1.52
Guarana plus caffeïn	5.40	5.68	5.56	5.81

Analyses of the pure alkaloids used to reinforce the above extracts gave the following results:

Percentages in a Series of Five Determinations.

Alkaloid.	Lowest.	Highest.	Average.	Amount Used.
Equal amounts of brucin and strychnin	1.47	1.60	1.52	1.50
Caffeïn	3.76	3.86	3.81	4.00
Atropin*	.92	1.00	.97	1.00

9. The above resumé of analytical data, shows, at least in the cases of the alkaloids subjected to the tests, a degree of uniformity, which warrants the adoption of the method for most purposes where the approximate valuation of fluid extracts is sought. The average loss and gain for the individual alkaloids as supplied by the above series of experiments, or still better by more extended series, could be used as factors to correct the unavoidable errors due to deterioration, or to increase of weight by treatment with chloroform, and bring the results very close to the truth.

In its present form the method certainly gives more reliable results than any other rapid process at the service of the chemist, and it is probable that more extended experiment will lead to modifications restricting still further the range of variation, as well as extending the range of application.

CINCINNATI, March 11, 1892.

*Means of single determinations.